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LSF bioproduction of citric acid by *Aspergillus candidus* F-569 exposed to diethyl sulphate

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Abstract: The efficacy of diethyl sulphate on liquid state fermentative bioproduction of citric acid by *Aspergillus parasiticus* F-112, *Aspergillus awamori* F-216, *Aspergillus foetidus* F-315, *Aspergillus flavus* F-418 and *Aspergillus candidus* F-569 has been assessed. It has been observed that the fungal strain *Aspergillus candidus* F-569 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e., diethyl sulphate has stimulatory effect on bioproduction of citric acid by *Aspergillus candidus* F-569 and enhances the yield of citric acid to an extent of 18.525% higher in comparison to control fermenter flasks, i.e., 10.074 g/100 ml under the optimized conditions.

(**Keywords:** Citric acid fermentation, diethyl sulphate (DES) and *Aspergillus candidus* F-569)

Introduction

Mutagenesis is described as the exposure or treatment of biological materialto a mutagen, i.e., a physical or chemical agent that raises the frequency ofmutation above the spontaneous rate[1]. Physical and chemical mutagens havebeen successfully used in plant breeding programs to artificially generategenetic variation for the development of new varieties with improved traitssuch as increased yield, earliness, reduced plant height, and resistance to disease[2]. In recent years, mutation induction became also a powerful tool forthe investigation of gene function and expression[3,4].

Mutation is a random event at the single cell level. Hence, the populationsize of the M1and M2generation must be adequate to cope with the workingobjective. This size depends on the probability to generate the desired varia-tion and on the inheritance of gene(s)[5]. Some thousands of seeds are usuallyneeded, being aware that the handling of such large populations requires effi-cient mass screening techniques[6].

The efficacy of different chemical mutagens in different fermentations process has been investigated by a number of workers[7-26]. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes[27-60].

Thus, from the above brief review it is evident that chemical mutagens are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the authors have studied the influence of diethyl sulphate (DES) on bioproduction of citric acid by *Aspergillus candidus* F-569

Experimental

The influence of diethyl sulphate on liquid state fermentative bioproduction of citric acid by *Aspergillus candidus* F-569

The composition of the production medium for liquid state fermentative bioproduction of citric acid by *Aspergillus candidus* F-569 has been prepared as follows: Molasses: 26.5%(w/v), NH_4NO_3: 0.350% , KH_2PO_4 : 0.375%, $MgSO_4.7H_2O$: 0.400%, pH : 2.2. The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter.

Table - 1
LSF bioproduction of citric acid by *Aspergillus candidus* F-569 exposed to diethyl sulphate

Concentration of mutagens used A X 10^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of citric acid in comparison to control after ten days
Control	5	4.147	8.286	-
(-) MUTAGEN	10	8.478	4.355	-
	15	5.123	4.245	-
1.0x10^{-4}M	5	4.168	8.265	-
(+) MUTAGEN	10	8.522	4.312	(+) 0.518
	15	5.148	4.218	-
2.0x10^{-4}M	5	4.205	8.228	-
(+) MUTAGEN	10	8.604	4.229	(+) 1.486
	15	5.194	4.118	-
3.0x10^{-4}M	5	4.238	8.195	-
(+) MUTAGEN	10	8.669	4.164	(+) 2.252
	15	5.235	4.084	-
4.0x10^{-4}M	5	4.329	8.108	-
(+) MUTAGEN	10	8.859	3.974	(+) 4.493
	15	5.348	3.868	-
5.0x10^{-4}M	5	4.408	8.025	-
(+) MUTAGEN	10	9.018	3.815	(+) 6.369
	15	5.445	3.710	-
6.0x10^{-4}M	5	4.532	7.890	-
(+) MUTAGEN	10	9.268	3.565	(+) 9.318
	15	5.599	3.456	-
7.0x10^{-4}M	5	4.665	7.758	-
(+) MUTAGEN	10	9.545	3.289	(+) 12.585
	15	5.763	3.185	-
8.0x10^{-4}M**	5	4.926	7.496	-
(+) MUTAGEN	10	10.074***	2.759	(+) 18.525
	15	6.086	2.668	-
9.0x10^{-4}M	5	4.511	7.922	-
(+) MUTAGEN	10	9.228	3.605	(+) 8.846
	15	5.573	3.514	-
10.0x10^{-4}M	5	4.283	8.152	-
(+) MUTAGEN	10	8.759	4.074	(+) 3.314
	15	5.292	4.002	-

* Each value represents mean of three trials ** Optimum concentration of mutagen used
*** Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 10 days. Experimental deviation (±) 1.5-3%

The above composition medium represents volume of a fermenter flask, i.e., "100ml" production medium of citric acid by *Aspergillus candidus* F-569. Now, the same production medium for bioproduction of citric acid by *Aspergillus candidus* F-569 was prepared for 99-fermenter flask, i.e.; each contained '100ml' of production medium.

The above 99-fermenter flasks were then arranged to 11-sets each comprising of 9-fermenter flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermenter flasks. The remaining 9-fermenter flasks out of 99-fermenter flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermenter flasks.

After preparing the above sets of fermenter flasks M/100 solution of diethyl sulphate was prepared and from the above mutagenic solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermenter flasks contained no mutagen. Now, the total volume in each fermenter flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of diethyl sulphate in 1st,2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x}$ M

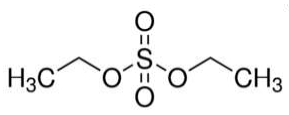
1.0×10^{-4}M to 10.0×10^{-4}M respectively.

i.e., Where A = amount of mutagen, in ml, i.e., 1.0 ml ..... to 10 ml, x =Molarity of the mutagen solution

The above fermenter flasks were then sterilized, cooled inoculated, incubated at 30^0C and analysed after 5, 10 and 15 days for citric acid[61] formed and molasses left unfermented[62].

Results and Discussion

The influence of diethyl sulphate



The data recorded in the table-1 shows the influence of diethyl sulphate on liquid state state fermentative bioproduction of citric acid by *Aspergillus candidus* F-569.

The data (vide table-1) reveals that the mutagen diethyl sulphate is stimulatory for citric acid fermentation process and enhances the yield of citric acid in comparison to control. The maximum yield of citric acid, i.e., 10.074 g/100 ml in the presence of 8.0×10^{-4}M concentration of diethyl sulphate has been observed which is 18.825% higher in comparison to control, i.e., 8.478 g/100 ml in 10 days of optimum incubation period.

It was interesting to note that the gradual addition of diethyl sulphate to the citric acid fermentative production medium gradually increases the yield of citric acid from 1.0×10^{-5}M to 8.0×10^{-5}M concentration of diethyl sulphate. It has been noticed further that on increasing the concentration of diethyl sulphate from 9.0×10^{-5}M to 10×10^{-5}M the production of citric acid was found to be in decreasing order.

However, maximum production (yield) of citric acid has been found at 8.0×10^{-5} M concentration of diethyl sulphate.

Thus, it is obvious that higher concentrations of diethyl sulphate has not been found inhibitory, yet after certain concentrations the influence of diethyl sulphate on liquid state fermentative bioproduction of citric acid by *Aspergillus candidus* F-569 has been found insignificant.

Therefore, higher concentrations of diethyl sulphate was not beneficial for the production of citric acid. However, liquid state fermentative bioproduction of citric acid by *Aspergillus candidus* F-569 at all the concentrations of diethyl sulphate used in the present investigation has been found greater than the control fermentor flasks.

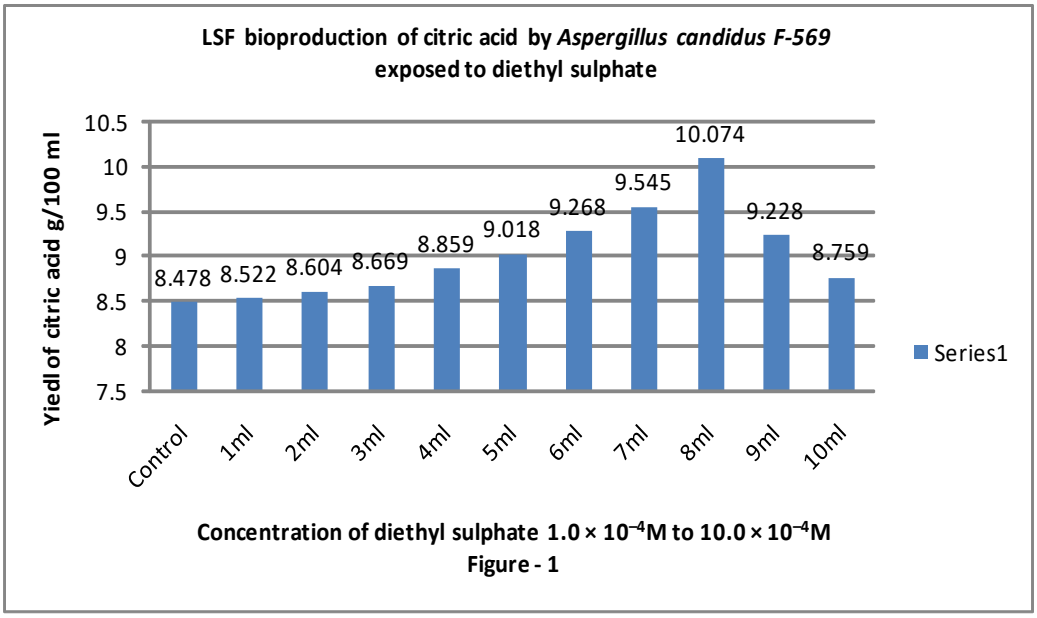


Figure - 1

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